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Bactericidal Activities of Plant Essential Oils and Some of Their Isolated Constituents against *Campylobacter jejuni, Escherichia coli, Listeria monocytogenes,* and *Salmonella enterica*

MENDEL FRIEDMAN,* PHILIP R. HENIKA, AND ROBERT E. MANDRELL

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 800 Buchanan Street, Albany, California 94710, USA

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ABSTRACT

An improved method of sample preparation was used in a microplate assay to evaluate the bactericidal activity levels of 96 essential oils and 23 oil compounds against *Campylobacter jejuni, Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella enterica* obtained from food and clinical sources. Bactericidal activity (BA50) was defined as the percentage of the sample in the assay mixture that resulted in a 50% decrease in CFU relative to a buffer control. Twenty-seven oils and 12 compounds were active against all four species of bacteria. The oils that were most active against *C. jejuni* (with BA50 values ranging from 0.003 to 0.009) were marigold, ginger root, jasmine, patchouli, gardenia, cedarwood, carrot seed, celery seed, mugwort, spikenard, and orange bitter oils; those that were most active against *E. coli* (with BA50 values ranging from 0.046 to 0.14) were oregano, thyme, cinnamon, palmarosa, bay leaf, clove bud, lemon grass, and allspice oils; those that were most active against *L. monocytogenes* (with BA50 values ranging from 0.057 to 0.092) were gardenia, cedarwood, bay leaf, clove bud, oregano, cinnamon, allspice, thyme, and patchouli oils; and those that were most active against *S. enterica* (with BA50 values ranging from 0.045 to 0.14) were thyme, oregano, cinnamon, clove bud, allspice, bay leaf, palmarosa, and marjoram oils. The oil compounds that were most active against *C. jejuni* (with BA50 values ranging from 0.003 to 0.034) were cinnamaldehyde, estragole, carvacrol, benzaldehyde, citral, thymol, eugenol, perillaldehyde, carvone R, and geranyl acetate; those that were most active against *E. coli* (with BA50 values ranging from 0.057 to 0.28) were carvacrol, cinnamaldehyde, thymol, eugenol, salicylaldehyde, geraniol, isoeugenol, citral, perillaldehyde, and estragole; those that were most active against *L. monocytogenes* (with BA50 values ranging from 0.019 to 0.43) were cinnamaldehyde, eugenol, thymol, carvacrol, citral, geraniol, perillaldehyde, carvone S, estragole, and salicylaldehyde; and those that were most active against *S. enterica* (with BA50 values ranging from 0.034 to 0.21) were thymol, cinnamaldehyde, carvacrol, eugenol, salicylaldehyde, geraniol, isoeugenol, terpineol, perillaldehyde, and estragole. The possible significance of these results with regard to food microbiology is discussed.

Food processors, food safety researchers, and regulatory agencies have been increasingly concerned with the growing number of foodborne illness outbreaks caused by some pathogens *(5, 11, 41).* The increasing antibiotic resistance of some pathogens that are associated with foodborne illness is another concern *(30, 33, 38, 39).* Therefore, there has been increasing interest in the development of new types of effective and nontoxic antimicrobial compounds.

Plant essential oils are a potentially useful source of antimicrobial compounds. Although numerous studies have been published on the antimicrobial activities of plant compounds against many different types of microbes, including foodborne pathogens *(3, 6, 9, 18, 26, 28, 37),* a review of the earlier literature *(13)* reveals that the results reported for these different studies are difficult to compare, presumably because of the different test methods, bacterial strains, and sources of antimicrobial samples used.

Three main factors can influence the results of a test of the antimicrobial activity of a plant oil: the composition and solubility of the oil, the microorganism, and the method of growing and enumerating the surviving bacteria *(43).* A unit commonly used in the measurement of antimicrobial activity is the diameter of the zone of inhibition of bacterial growth on solid medium. For plant essential oil samples, the zone of inhibition will depend on the ability of oil to diffuse uniformly through an agar medium and the effect on bacteria of oil vapors that may be released. Other variables in tests of plant antimicrobial compounds include the presence of two or more active components that may interact antagonistically, additively, or synergistically at low concentrations; changes (resulting from the partitioning of active components between the lipid and the aqueous phases) in the antimicrobial activity of oils in complex test samples (e.g., food) compared with the activity of the oils alone; and substances present in complex sample reaction mixtures that may stimulate or inhibit the growth of the test microorganisms independent of the test sample. It is important to standardize test methods and to evaluate factors

* Author for correspondence. Tel: 510-559-5615; Fax: 510-559-5777; E-mail: mfried@pw.usda.gov.

TABLE 1. *Bacterial strains used in this study*

Strain[a]	Original strain designation	Species/serotype	Other information[b]
RM1239	96A13466	*E. coli* O157:H7	CDHS; isolated from human feces, associated with consumption of apple juice
RM1272	ATCC 43895	*E. coli* O157:H7	Isolated from hamburger, SLTI/SLTII
RM1275	94A8338	*E. coli* O157:H7	CDHS; isolated from human feces, associated with consumption of salami, SLTI/SLTII
RM1273	ATCC 343888	*E. coli* O157:NM	Isolated from human feces
RM1277	94A3553	*E. coli* O157:H7	CDHS; isolated from human feces, SLTI/SLTII
RM1484	SEA13B88	*E. coli* O157:H7	FDA; isolated from apple juice, associated with outbreak
RM1253	96A5291	*S. enterica* serovar Meleagridis	CDHS; isolated from human feces, associated with outbreak
RM1254	96A7406	*S. enterica* serovar Meleagridis[c]	CDHS; isolated from alfalfa sprouts, associated with outbreak
RM1309	MH136	*S. enterica* serovar Hadar	Isolated from ground turkey
RM1655	96E01153C-TX	*S. enterica* serovar Newport	CDSH; isolated from alfalfa seeds, associated with outbreak
RM1046	ATCC 43430	*C. jejuni*	Penner serotype O:2, isolated from calf feces
RM1221	This study	*C. jejuni*	Isolated from retail market chicken breast (*31*)
RM1230	This study	*C. jejuni*	Isolated from retail market chicken breast
RM1274	This study	*C. jejuni*	Isolated from retail market chicken breast
RM2199	F2379	*L. monocytogenes*	UCB; isolated from cheese associated with outbreak (*1*)
RM2388	This study	*L. monocytogenes*	Isolated from retail mint herb, serotype 1

[a] Produce Safety and Microbiology Research Unit strain file designation.
[b] CDHS, California Department of Health Services Microbial Diseases Laboratory Branch; ATCC, American Type Culture Collection; FDA, Food and Drug Administration; SLTI/SLTII, positive for Shiga-like toxin types I and II; UCB, University of California, Berkeley.
[c] Food isolate matched human isolate RM1253.

that influence the potencies of antimicrobial agents in order to enable comparisons of results obtained in different studies *(24)*.

The general objective of this study was to screen a broad variety of naturally occurring and potentially food-compatible plant-derived oils and oil compounds for their antimicrobial activities against an epidemiologically relevant group of four species of bacterial foodborne pathogens. The specific objectives of this study were (i) to develop an improved sample preparation technique and an assay for testing the levels of bactericidal activity of plant essential oils and purified plant compounds present in essential oils against different strains of four major foodborne pathogens; (ii) to develop a standard parameter for comparing dose-response data; (iii) to compare the bactericidal activities of 96 plant essential oils and 23 purified oil compounds; and (iv) to identify structural features in the oil constituents that may be responsible for these bactericidal activities. The usefulness of the findings of this study in the protection of foods against contamination is also discussed.

MATERIALS AND METHODS

Test compounds. The following plant essential oils were purchased from Yerba Buena Company (Berkeley, Calif.): allspice, almond bitter, almond sweet, aloe vera, anise seed, anise star, apricot, balsam Peru, basil, bay leaf, benzoin gum, bergamot, birch, cajeput, caraway, cardamon, carrot seed, cedarwood, celery seed, chamomile Roman, cinnamon bark, cinnamon cassia, cinnamon leaf, citronella, clove bud, coriander, cumin seed, cypress, dill weed, elemi, eucalyptus, evening primrose, fennel seed, fir needle balsam, fir needle Siberian, frankincense, gardenia, ginger root, grape seed, hazelnut, helichrysum, hyssop, jasmine, jojoba, juniper berry, lavender, lavender spike, lemon, lemon grass, lemon verbena, lime, marigold calendula, marigold taegetes, margoram, mugwort, myrrh gum, myrtle, nutmeg, oakmoss, orange bitter, orange mandarin, orange neroli blossom, orange sweet, oregano origanum, oregano Spanish, palmarosa, patchouli, pennyroyal, pepper black, peppermint, petitgrain, pine needle, ravensara, rose damask, rose French, rose geranium, rosemary, rosewood, sage clary; sage white dalmatian, sage white desert, sandalwood Indian mysore, sassafras, sesame, spearmint, spikenard, spruce, tangerine, tarragon, tea tree, thuja, thyme, tuberose, vanilla oleo resin, wintergreen, wormwood, and ylang ylang. These oils were considered 100% pure.

The following chemicals were obtained from Sigma (St. Louis, Mo.): (−)-*trans*-anethole, benzaldehyde, (−)-bornyl acetate, carvacrol, chloramphenicol, cineole (eucalyptol), citral, *R*-(+)-citronellal, *S*-(−)-citronellal, eugenol, gentamycin, geraniol, geranyl acetate, (−)-menthol, salicylaldehyde, terpineol (mixed isomers), and thymol. Additional chemicals, obtained from Aldrich (Milwaukee, Wis.), were estragole (4-allylanisole), *R*-(−)-carvone, *S*-(+)-carvone, *trans*-cinnamaldehyde, isoeugenol (mixture of *cis* and *trans* isomers), (+)-limonene, linalool, and perillaldehyde. The purity levels of these compounds ranged from 92 to 99% according to the manufacturer.

Bacterial strains. Strain numbers, the sources of the strains, and other information regarding the bacteria used in this study are provided in Table 1. All of the strains used in this study are designated by their Produce Safety and Microbiology Research Unit strain file numbers (Table 1).

Growth of bacteria. *Escherichia coli* and *Salmonella enterica* strains stored in vials at −80°C were streaked on Luria-Bertani (LB) agar plates (Difco Laboratories, Sparks, Md.) after thawing. These plates were then incubated at 37°C for 18 to 24 h. A few isolated colonies from each plate were harvested with a sterile loop and suspended in 5 ml of LB broth in a sterile 15-ml plastic

tube. The tube was capped tightly and incubated with gentle shaking (150 rpm) at 37°C for 18 h. The procedure was the same for *Listeria monocytogenes* strains except that brain heart infusion agar was substituted for LB agar. *Campylobacter jejuni* strains stored in vials at −80°C were plated on brucella agar containing iron supplements *(31).* Plates streaked with *C. jejuni* were incubated in sealed bags filled with a microaerophilic gas mixture (5% O_2, 10% CO_2, and 85% N_2) for 18 to 19 h at 42°C. The bags were filled with gas, the gas was expelled three times, and then the bags were sealed tightly to ensure the proper atmosphere. Streaked plates, rather than broth cultures, were used as the source of *C. jejuni.*

Preparation of plant essential oils and purified compounds for bactericidal assay. Many of the plant essential oils and many of the compounds purified from essential oils are immiscible in the aqueous buffers used in bactericidal assays. A simplified shaking method was developed to prepare 1% suspensions of oil in aqueous phosphate-saline buffer (50 mM phosphate-buffered saline [PBS; pH 7]); this method involved vigorous shaking of the sample followed by dilutions of each suspension in PBS, with each dilution of sample being mixed carefully. Repetitive studies showed that this method yielded reproducible results even with samples that were immiscible in PBS.

It was noted during suspension preparation that some oils separated more slowly than others did. The shaking of a 1% suspension of cinnamaldehyde resulted in a grayish, milky oil suspension; this separation was considered ''slow.'' For a 1% suspension of oregano origanum oil, shaking resulted in a rapid pooling of yellow oil droplets on the surface; this separation was considered ''fast.'' An aliquot (200 μl) of oil suspension was drawn immediately after shaking and added to 400 μl of PBS buffer for a 1:3 dilution. The dilution was shaken for 10 s before an aliquot was added to the next tube. Samples were serially diluted five times, for six dilutions.

Two of the compounds tested (menthol and thymol) were solids. Approximately 10 to 15 mg of either compound was added to a 1.9-ml sterile snap-cap polypropylene microcentrifuge tube, suspended in 1 ml PBS buffer, and vortexed for 1 min. Samples were warmed in a microwave oven for 4 s at high power, vortexed for 30 s, and then shaken for 10 s. Menthol and thymol formed oily gray suspensions under these conditions.

In most cases, the shaking procedure was performed with a 10-ml Erlenmeyer flask containing 4.95 ml of PBS and 50 μl of oil (1% stock solution). The flask was shaken by hand for approximately 5 s and then tilted to fill the neck for further mixing. After this procedure had been repeated three times, 0.5 ml of the suspension was transferred into a 1.9-ml sterile plastic tube and dilutions were made. Initially, soluble standard antibiotics were included in all experiments as positive controls (gentamycin for *E. coli, S. enterica,* and *C. jejuni* and chloramphenicol for *L. monocytogenes*). To mimic oil suspension behavior, *trans*-cinnamaldehyde was used in repeated experiments for the first three bacteria. We chose the shaking method to simulate what the consumer might do with separated oils (e.g., shaking a salad dressing).

Preparation of bacteria for bactericidal assay. A 1-ml sample of a broth culture of a strain of *E. coli, S. enterica,* or *L. monocytogenes* was added to a 1.9-ml microfuge tube, and the bacteria were pelleted by centrifugation in a microfuge at 12,000 rpm for 30 s. After the removal of the supernatant, 1 ml of sterile PBS was added to the pellet, and the pellet was resuspended by gentle aspiration in and out of a transfer pipette. The optical density (OD) of the resuspended pellet was determined with a spectrophotometer set at 620 nm. The OD_{620} of the sample was adjusted to approximately 0.8 to 0.9 with the addition of PBS. Ten microliters of the diluted sample was added to 990 μl of PBS (1:100), and then the 1:100 sample was diluted in PBS to 1:28,000 for *E. coli* or *L. monocytogenes* and to 1:38,000 for *S. enterica.* These dilutions resulted in approximately 1,500 to 2,000 bacteria per 50-μl sample. These ODs produced 60 to 200 CFU per lane.

Isolated colonies of *C. jejuni* on plates after 18 to 19 h of incubation as described above were harvested with a sterile loop and then transferred to 1 ml of PBS in a plastic cuvette. The cells were suspended by gentle aspiration in and out of a transfer pipette 10 times, and the OD_{620} value was adjusted to 1.2 to 1.5 with PBS. The bacteria were pelleted by centrifugation in a microfuge at 12,000 rpm for 30 s. The pellet was resuspended with 1 ml of sterile PBS and aspirated 10 times, and the OD_{620} was then read in the 1.0-to-1.3 range. Ten microliters of the diluted sample was added to 990 μl of PBS (1:100), and then the 1:100 sample was diluted in PBS to a final dilution of 1:20,000.

Bactericidal assay. The assay to assess the antimicrobial activities of plant compounds was developed through the modification of a microtiter plate bactericidal assay described previously *(22).* The assay reaction mixture consisted of PBS (50 mM sodium phosphate, 150 mM NaCl [pH 7.0]), the test compound, and the bacteria. The samples were prepared in sterile 96-well tissue culture microtiter plates (Nunc, Inc). Each dilution was mixed by shaking, a 100-μl sample in PBS was added to the well, and then 50 μl of the diluted bacteria was added.

The microtiter plates were incubated with gentle shaking (150 rpm) at 37°C (42°C for *C. jejuni*) for 1 h. For time course experiments, a microtiter plate, along with a matched control and dilutions of test substances, was prepared for each incubation time studied (0, 10, 20, 40, 80, 160, and 320 min). After the removal of a microtiter plate from the incubator, it took 3 to 4 min to plate a control and three test substances.

Following incubation, a 20-μl aliquot from each well was spotted at the top of a square plate containing LB agar (for *E. coli* and *S. enterica*), brain heart infusion agar (for *L. monocytogenes*), or iron-supplemented brucella agar (for *C. jejuni*). The plate was tilted and tapped gently to facilitate the movement of the liquid to the bottom of the plate. A 20-μl volume was used so that samples would not mix; six 20-μl samples evenly spaced across the top could accommodate each of six dilutions for test samples or six controls. There were approximately 200 cells in the spotted 50-μl sample.

Plates were placed uncovered in a biohood until the sample liquid dried (ca. 10 min); then the plates were covered and *E. coli, S. enterica,* and *L. monocytogenes* plates were incubated overnight at 37°C. *C. jejuni* plates were placed in sealable bags, and the bags were filled with gas (5% O_2, 10% CO_2, and 85% N_2) as described above and incubated at 42°C.

CFU of *E. coli* and *S. enterica* on LB agar and those of *L. monocytogenes* on brain heart infusion agar were visible after 18 to 24 h and were counted. CFU of *C. jejuni* on iron-supplemented brucella agar were small but countable after 24 h. In a typical experiment with a 96-well microtiter plate, 50 μl of bacteria was added to 6 wells containing PBS (negative control), 6 wells containing doses of a positive control, and 6 wells each for doses of 14 test compounds. The experiments were performed in duplicate with two separately prepared bacterial suspensions for each strain. CFU for each streak were enumerated with a colony counter. Usually, 60 to 200 CFU were present in the negative control. Positive control values were obtained with a dilution series of gentamycin, *trans*-cinnamaldehyde, or chloramphenicol.

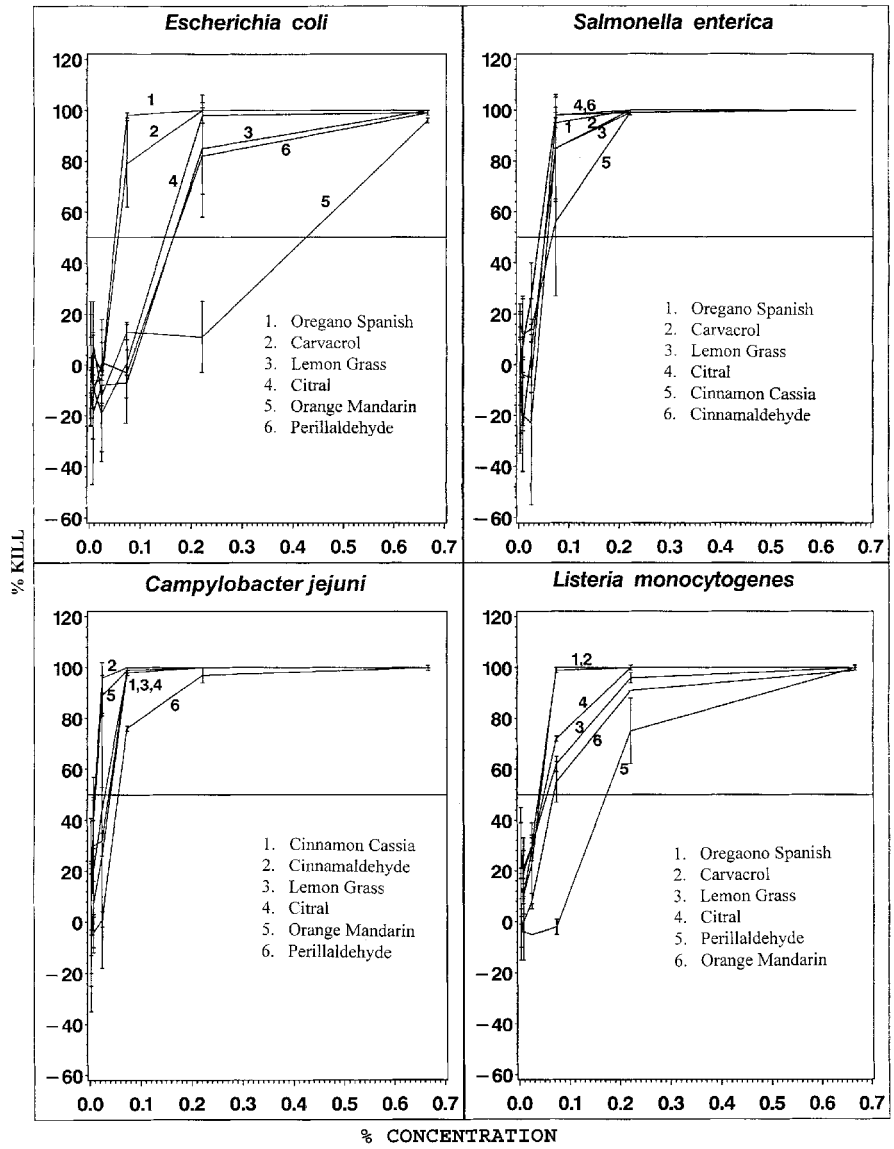

FIGURE 1. *Dose-response plots of concentration percentage versus kill percentage for plant essential oils and oil compounds against* E. coli *O157:H7 (strain RM1484),* S. enterica *(strain RM1309),* C. jejuni *(strain RM1221), and* L. monocytogenes *(strain RM2199). Vertical bars indicate standard deviations from the mean.*

Effect of incubation time on antimicrobial activity. To compare the sensitivities of the bacteria at different incubation times, each of the prototype strains of *E. coli* and *S. enterica* was assayed with cinnamon oil, oregano origanum oil, and eugenol (a constituent of clove oil) at incubation times ranging from 10 to 320 min (data not shown). The results for both *E. coli* and *S. enterica* indicated increases in bactericidal activity for the first 60 min of incubation for each of the samples, followed by a plateau in activity during the next 60 to 120 min. Because these studies demonstrated that the antimicrobial activity levels of the most active compounds were near a maximum at approximately 60 min, this incubation time was selected for subsequent experiments.

BA50 values. The numbers of CFU counted for the six concentrations of the negative controls, the positive controls, and the compounds were transferred to a Microsoft Excel 8.0 spreadsheet. The number of CFU at each dilution was matched with the average negative control value to determine the percentage of bacteria killed per well. The percentage of the test compound in the well and the percentage of bacteria killed were plotted graphically by linear regression, and the percentage of the test compound in the mixture resulting in a 50% decrease in the number of CFU (BA50) was determined (Fig. 1). BA50 was chosen as the measure of bactericidal activity because it can be obtained from the linear part of the dose-response plots of a dilution series, although other measures of bactericidal activities, such as MIC for 99.9% kill, can be obtained from the full dilution series (dose-response plots) shown in Figure 1. BA50 was calculated to determine the relative potencies and the ranking of bactericidal activities. The lower the BA50 value, the higher the bactericidal activity level. Reciprocal BA50 values (1/BA50) were also calculated to relate the BA50 value directly with bactericidal activity.

RESULTS

Dose-response plots to determine BA50 values. Bactericidal activity is presented in Figure 1 as the kill percentage plotted against the test sample concentration percentage. All BA50 values were determined from such plots. The results are also shown in terms of log concentration percentages in Figure 2. Since the doses used are equally spaced and become additive, log concentration percentage plots facilitate the visualization of subtle differences in the activities of compounds.

Selected samples were tested against the four prototype strains representing each species. Each of the strains tested was effectively killed by many of the oils and oil com-

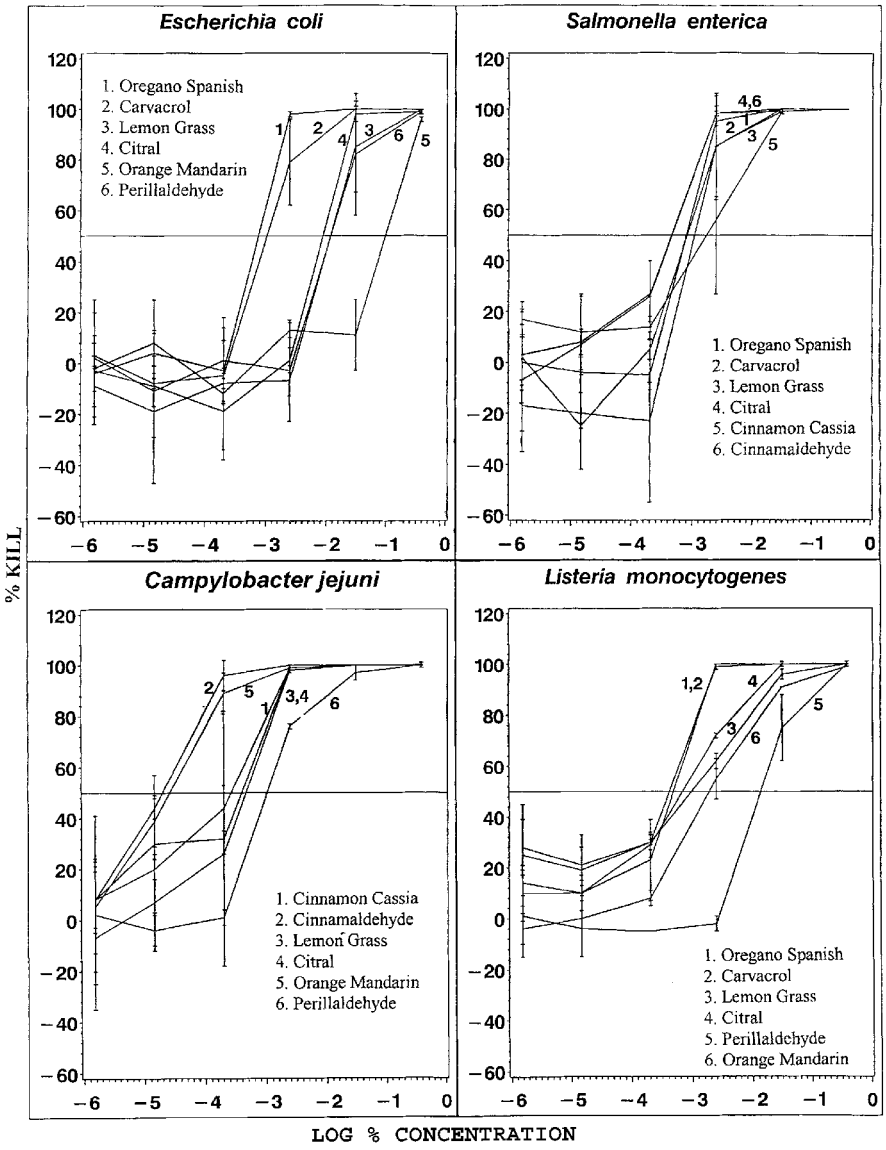

FIGURE 2. *Dose-response plots of log concentration percentage versus kill percentage for plant essential oils and oil compounds against* E. coli *O157:H7 (strain RM1484),* S. enterica *(strain RM1309),* C. jejuni *(strain RM1221), and* L. monocytogenes *(strain RM2199). Vertical bars indicate standard deviations from the mean.*

pounds tested at concentrations of >0.7%. For example, *E. coli, S. enterica,* and *L. monocytogenes* were killed at a rate of >90% by oregano Spanish concentrations of <0.1%. All four species were killed at a rate of >90% by a lemon grass oil concentration of <0.2%. The region of the dose-response curve between kill rates of 20 and 70% for each of the four strains was relatively linear for the range of concentrations plotted; therefore, the concentration of a compound that resulted in a kill rate of 50% (BA50) was selected as the basis for a comparison of the bactericidal activities of other compounds.

Bactericidal activities of cinnamon bark and oregano origanum oils and eugenol against multiple strains of *E. coli, S. enterica, C. jejuni,* and *L. monocytogenes.* Two plant essential oils (cinnamon bark and oregano origanum oils) and one purified plant compound (eugenol) were tested for bactericidal activity against six strains of *E. coli* (five O157:H7 and one O157:NM), four strains each of *S. enterica* and *C. jejuni,* and two strains of *L. monocytogenes.* The results of the test are summarized in Table 2. BA50 values for oregano origanum oil, cinnamon bark oil, and eugenol for all 16 strains ranged from 0.02 to 0.10, from 0.037 to 0.18, and from 0.011 to 0.10, respectively. Generally, the BA50 values for a compound for different strains of a single species were less than twofold different, with the exception that *E. coli* O157:H7 strain RM1277 (BA50 = 0.019) was more sensitive to eugenol than were the other *E. coli* strains tested (with BA50 values ranging from 0.052 to 0.10). *C. jejuni* strains were more sensitive to the three compounds (with BA50 values ranging from 0.011 to 0.08) than were strains of the other species tested (with BA50 values ranging from 0.019 to 0.18).

Bactericidal activities of 96 essential oils against single strains of *E. coli, S. enterica,* and *C. jejuni* and two strains of *L. monocytogenes.* Individual strains of *E. coli, S. enterica,* and *C. jejuni* and two strains of *L. monocytogenes* were tested for their susceptibility to 96 different plant essential oils and three samples included as positive controls (chloramphenicol, gentamycin, and cinnamaldehyde). A summary of the results of these experiments is shown in Table 3. The five oils that were most active against *E. coli* RM1484 were oregano (Spanish), thyme,

TABLE 2. *BA50 values for oregano oil, cinnamon oil, and eugenol with different strains of* E. coli *O157:H7,* S. enterica, C. jejuni, *and* L. monocytogenes[a]

Strain	BA50 value for:		
	Oregano origanum	Cinnamon bark	Eugenol
E. coli			
RM1484 (prototype strain)	0.05 ± 0.01	0.15 ± 0.04	0.10 ± 0.03
RM1239	0.066	0.18	0.060
RM1272	0.069	0.18	0.082
RM1273	0.028	0.14	0.052
RM1275	0.063	0.17	0.058
RM1277	0.052	0.15	0.019
S. enterica			
RM1309 (prototype strain)	0.06 ± 0.02	0.12 ± 0.01	0.09 ± 0.03
RM1252	0.10	0.12	0.093
RM1254	0.092	0.12	0.10
RM1655	0.085	0.14	0.099
C. jejuni			
RM1221 (prototype strain)	0.02 ± 0.01	0.04 ± 0.03	0.02 ± 0.01
RM1046	0.025	0.037	0.011
RM1230	0.025	0.043	0.023
RM1274	0.031	0.080	0.023
L. monocytogenes			
RM2199 (prototype strain)	0.08 ± 0.01	0.09 ± 0.004	0.06 ± 0.05
RM2388	0.04 ± 0.03	0.08 ± 0.005	0.08 ± 0.001

[a] The values shown for prototype strains for *E. coli, S. enterica,* and *C. jejuni* represent averages ± standard deviations for at least three experiments. The values shown for both *L. monocytogenes* strains (RM2199 and RM2388) represent the averages ± standard deviations for two separate experiments.

oregano (origanum), cinnamon (cassia), and palmarosa (with BA50 values ranging from 0.045 to 0.12). The five oils that were most active against *S. enterica* RM1309 were thyme, oregano Spanish, oregano origanum, cinnamon cassia, and cinnamon leaf (with BA50 values ranging from 0.045 to 0.08). The five oils that were most active against *C. jejuni* RM1221 were marigold taegetes, ginger root, jasmine, patchouli, and gardenia (with BA50 values ranging from 0.003 to 0.007). The five oils that were most active against *L. monocytogenes* RM2199 were gardenia, cedarwood, bay leaf, oregano Spanish, and clove bud (with BA50 values ranging from 0.057 to 0.074). The five oils that were most active against *L. monocytogenes* RM2388 were spikenard, cedarwood, patchouli, gardenia, and orange sweet (with BA50 values ranging from 0.02 to 0.04). The prototype *C. jejuni* strain (RM1221) was again more sensitive to the oils than were the other strains. Eighty-one of the oils tested had BA50 values of <0.2 for *C. jejuni,* compared with 12, 13, 22, and 35 oils with BA50 values of <0.2 for *E. coli* RM1484, *S. enterica* RM1309, *L. monocytogenes* RM2199, and *L. monocytogenes* RM2388, respectively (Table 3). Oils with BA50 values of ≤ 0.12 for each of the four species tested were oregano Spanish, oregano origanum, thyme, bay leaf, clove bud, allspice, cinnamon leaf, cinnamon bark, cinnamon cassia, and lemon grass.

Seven of the oils had BA50 values that differed by more than threefold (helichrysum, lemon verbena, lime, pennyroyal, spikenard, tangerine, and wormwood) for the two *L. monocytogenes* strains (Table 3). In addition, 17 other oils were inactive against *L. monocytogenes* RM2199 (BA50 > 0.67) but were active against *L. monocytogenes* RM2388 (with BA50 values ranging from 0.069 to 0.66).

Bactericidal activities of oil compounds. The prototype strains were also tested for their susceptibility to 23 aldehydes, phenols, and alcohols known to be constituents of plant essential oils (see "Materials and Methods"). The BA50 values for these compounds are shown in Table 4, the chemical structures of the compounds are shown in Figure 3, and a summary of the relative activities of 39 oils and oil compounds that were active against all bacterial strains is presented in Table 5. The prototype *C. jejuni* strain remained the most sensitive of the species and was sensitive to many of the oil compounds. Twelve compounds had BA50 values of <0.05 for *C. jejuni* RM1221, compared with 0, 2, 1, and 1 compounds with BA50 values of <0.05 for *E. coli* RM1484, *S. enterica* RM1309, *L. monocytogenes* RM2199, and *L. monocytogenes* RM2388, respectively. The compounds that were most active against the *E. coli, S. enterica,* and *L. monocytogenes* strains were cinnamaldehyde (with BA50 values ranging from 0.008 to 0.057), thymol (with BA50 values ranging from 0.034 to 0.077), carvacrol (with BA50 values ranging from 0.011 to 0.086), and eugenol (with BA50 values ranging from 0.022 to 0.11) (Tables 4 and 5). The most active compound for all of the strains was cinnamaldehyde (with BA50 values ranging from 0.0028 to 0.057) (Tables 4 and 5).

TABLE 3. *BA50 values for essential oils against* E. coli *O157:H7,* S. enterica, C. jejuni, *and* L. monocytogenes[a]

Oil	BA50 value for: *E. coli* RM1484	*S. enterica* RM1309	*C. jejuni* RM1221	*L. monocytogenes* RM2199	*L monocytogenes* RM2388	Suspension[b]
Allspice	0.14 ± 0.02	0.13 ± 0.01	0.23 ± 0.003	0.089 ± 0.02	0.076 ± 0.005	gn, r
Almond bitter	>0.67 (1.1)	>0.67 (1.1)	0.042 ± 0.06[c]	>0.67 (0.84)	0.25 ± 0.02	gy
Almond sweet	>0.67	>0.67	>0.67	>0.67	>0.67	gy
Aloe vera	>0.67	>0.67	>0.67	>0.67	>0.67	gy
Anise seed	>0.67 (1.3)	>0.67 (1.3)	0.10 ± 0.02[c]	>0.67	0.31 ± 0.02	gy, r
Anise star	>0.67	>0.67	0.22 ± 0.10	>0.67	>0.67	gy
Apricot	>0.67	>0.67	>0.67	>0.67	>0.67	gy
Balsam Peru	>0.67	>0.67	0.15 ± 0.03	>0.67	>0.67	gy, gn
Basil	0.41 ± 0.04	0.42 ± 0.02	0.023 ± 0.01	0.089 ± 0.02	0.12 ± 0.02	gy
Bay leaf	0.13 ± 0.02	0.13 ± 0.02	0.034 ± 0.01[c]	0.070 ± 0.02	0.073 ± 0.001	gn, r
Benzoin gum	>0.67	>0.67	0.031 ± 0.005	0.35 ± 0.04	>0.67	gy, gn
Bergamot	0.41[d]	0.51 ± 0.06	0.081 ± 0.05	>0.67	>0.67 (0.87)	gy
Birch	>0.67	>0.67	0.29 ± 0.15	>0.67	>0.67	gy
Cajeput	0.19 ± 0.02	0.36 ± 0.09	0.065 ± 0.04	>0.67 (0.71)	>0.67 (0.92)	gy
Caraway	0.46 ± 0.04	0.47 ± 0.09	0.029 ± 0.004[c]	0.33 ± 0.01	0.24 ± 0.01	gy
Cardamon	>0.67	>0.67	0.022 ± 0.01	0.58 ± 0.14[e]	0.40 ± 0.01	gy
Carrot seed	>0.67	>0.67	0.0078 ± 0.002	0.15 ± 0.03[f]	0.052 ± 0.01	gy, r
Cedarwood	>0.67	>0.67	0.0075 ± 0.001	0.067 ± 0.06[f]	0.028 ± 0.003	gy
Celery seed	>0.67	>0.67	0.0085 ± 0.001	0.29 ± 0.13	0.13 ± 0.001	gy, m
Chamomile Roman	>0.67	>0.67	0.022 ± 0.004	0.32 ± 0.09[f]	0.29 ± 0.12	gy, gn
Chloramphenicol	—	—	—	0.020 ± 0.01[g]	0.016 ± 0.003[h]	s
Cinnamaldehyde	0.057 ± 0.034[i]	0.033 ± 0.14[j]	0.0028 ± 0.002[k]	0.019 ± 0.01	0.008 ± 0.001	gy, m
Cinnamon bark	0.18 ± 0.02	0.14 ± 0.01	0.021 ± 0.004[c]	0.085 ± 0.004	0.079 ± 0.005	gy
Cinnamon cassia	0.11 ± 0.04[f]	0.066 ± 0.036[c]	0.014 ± 0.01[c]	0.19 ± 0.08	0.15 ± 0.04	gy, gn, m
Cinnamon leaf	0.13 ± 0.01	0.084 ± 0.048[c]	0.027 ± 0.002	0.087 ± 0.03	0.090 ± 0.004	gn, m
Citronella	0.41 ± 0.03	0.49 ± 0.11	0.086 ± 0.01	0.40 ± 0.16[l]	0.18 ± 0.13[f]	gy
Clove bud	0.13 ± 0.01[f]	0.13 ± 0.02[c]	0.016 ± 0.01	0.074 ± 0.06	0.092 ± 0.005	gn, r, m
Coriander	0.40 ± 0.04	0.48 ± 0.04	0.081 ± 0.03	0.665 ± 0.04	0.50 ± 0.18	gy
Cumin seed	0.30 ± 0.21	0.36 ± 0.02	0.099 ± 0.005	0.37 ± 0.06	0.25 ± 0.06	gn, y, r
Cypress	>0.67	>0.67	0.084 ± 0.01	0.11 ± 0.08[f]	0.27 ± 0.11	gy
Dill weed	0.40 ± 0.07	0.48 ± 0.04	0.087 ± 0.01	>0.67 (0.82)	0.66 ± 0.20	gn, y, r
Elemi	0.40 ± 0.07	0.44 ± 0.08	0.011 ± 0.005	0.26 ± 0.04	0.22 ± 0.01	gy
Eucalyptus	>0.67	>0.67	0.028 ± 0.01	>0.67 (0.78)	0.34 ± 0.04	gy
Evening primrose	>0.67	>0.67	0.32 ± 0.01	>0.67	>0.67	gy
Fennel seed	0.56 ± 0.10[e]	0.38 ± 0.21[e]	0.10 ± 0.01	>0.67	>0.67 (1.1)	gy
Fir needle balsam	>0.67	>0.67	0.047 ± 0.04[f]	0.37 ± 0.06	0.17 ± 0.002	gy
Fir needle Siberian	0.48 ± 0.04[f]	0.61 ± 0.04[f]	0.014 ± 0.01	0.13 ± 0.02	0.082 ± 0.02	gy

TABLE 3. *Continued*

Oil	BA50 value for: *E. coli* RM1484	*S. enterica* RM1309	*C. jejuni* RM1221	*L. monocytogenes* RM2199	*L monocytogenes* RM2388	Suspension[b]
Frankincense	>0.67	>0.67	0.025 ± 0.003	>0.67	0.27 ± 0.09	gy
Gardenia	>0.67 (0.69)	>0.67	0.007 ± 0.003[c]	0.057 ± 0.004[f]	0.038 ± 0.03	y, r, m
Gentamycin	0.00012 ± 0.00003[m]	0.00006 ± 0.00004[n]	0.00006 ± 0.00002[o]	—	—	s
Ginger root	>0.67	>0.67	0.005 ± 0.005	0.50 ± 0.06[e]	0.23 ± 0.16	gy
Grape seed	>0.67	>0.67	0.63[d]	>0.67	>0.67 (0.98)	gy
Hazelnut	>0.67	>0.67	>0.67	>0.67	>0.67 (1.2)	gy
Helichrysum	>0.67	>0.67	0.10 ± 0.11	0.50 ± 0.01	0.09 ± 0.03	gn, gy, m
Hyssop	0.57 ± 0.06	0.41 ± 0.02	0.096 ± 0.02	0.33 ± 0.04	0.18 ± 0.01	gy
Jasmine	>0.67	>0.67	0.006 ± 0.003	0.30 ± 0.11	0.36 ± 0.06	o, r
Jojoba	>0.67	>0.67	>0.67	>0.67	>0.67	gy
Juniper berry	>0.67	0.43 ± 0.04	0.034 ± 0.01	0.33 ± 0.14[f]	0.19 ± 0.10[f]	gy
Lavender	0.41 ± 0.04	0.41 ± 0.03	0.061 ± 0.05	0.48 ± 0.04	0.34 ± 0.03	y, gn
Lavender spike	0.43 ± 0.01	0.28 ± 0.11[c]	0.083 ± 0.02	>0.67 (0.71)	>0.67 (0.77)	gy
Lemon	>0.67	>0.67	0.045 ± 0.05[c]	0.35 ± 0.12[f]	0.22 ± 0.07[f]	gy, m
Lemon grass	0.14 ± 0.05[f]	0.16 ± 0.04	0.018 ± 0.011[c]	0.12 ± 0.02	0.12 ± 0.04	gy
Lemon verbena	0.49 ± 0.09	>0.67 (1.5)	0.012 ± 0.01[c]	0.24 ± 0.13	0.086 ± 0.04	gn, gy, m
Lime	0.50 ± 0.03	>0.67 (1.0)	0.044 ± 0.001[f]	0.22 ± 0.04	0.056 ± 0.02	gy
Marigold calendula	>0.67	>0.67	0.02 ± 0.01	0.37 ± 0.09	0.35 ± 0.25[f]	g, o, r
Marigold taegetes	>0.67	0.40 ± 0.04	0.003 ± 0	>0.67	0.18 ± 0.03	gn, y
Marjoram	0.43 ± 0.02	0.14 ± 0.01	0.026 ± 0.001	>0.67 (1.3)	>0.67 (0.99)	gy
Mugwort	0.57 ± 0.09	0.40 ± 0.05[e]	0.009 ± 0.0003	>0.67 (1.1)	0.56 ± 0.06	gy
Myrrh gum	>0.67	>0.67	0.026 ± 0.01	>0.67	0.069 ± 0.003	gn, gy
Myrtle	>0.67	>0.67	0.23 ± 0.13	0.11 ± 0.03	0.10 ± 0.02	gn, gy
Nutmeg	0.55 ± 0.16[c]	0.44 ± 0.03	0.18 ± 0.14	0.27 ± 0.02	0.20 ± 0	gy
Oakmoss	>0.67	>0.67	0.22 ± 0.13	0.37 ± 0.05	0.40 ± 0.28	y, gn, r
Orange bitter	0.47 ± 0.06[f]	>0.67 (1.0)	0.009 ± 0.003[f]	0.095 ± 0.004	0.075 ± 0.005	gy
Orange mandarin	0.41 ± 0.15[f]	0.64 ± 0.47[f]	0.010 ± 0.01[f]	0.18 ± 0.05	0.10 ± 0.02	gy
Orange neroli blossom	0.45 ± 0.01	>0.67 (0.91)	0.016 ± 0.01[f]	0.12 ± 0.01	0.21 ± 0.08	gn, gy
Orange sweet	>0.67	>0.67	0.077 ± 0.06	0.097 ± 0.05	0.040 ± 0.01	gy
Orégano origanum	0.048 ± 0.004	0.050 ± 0.004	0.019 ± 0.01[f]	0.078 ± 0.01	0.098 ± 0.118[f]	y, r
Oregano Spanish	0.046 ± 0.004	0.049 ± 0.001	0.011 ± 0.01[f]	0.074 ± 0.01	0.077 ± 0.01	y, r
Palmarosa	0.12 ± 0.01	0.14 ± 0.001	0.067 ± 0.04	0.17 ± 0.02	0.27 ± 0.01	gy
Patchouli	>0.67	>0.67	0.007 ± 0.001	0.092 ± 0.05	0.029 ± 0.01	gn, y, r
Pennyroyal	0.25 ± 0.09	0.41 ± 0.02	0.12 ± 0.06	>0.67 (0.84)	0.53 ± 0.21	gy
Pepper black	>0.67	>0.67	0.034 ± 0.01	>0.67 (0.86)	0.13 ± 0.06[c]	gy
Peppermint	0.47 ± 0.07	0.53 ± 0.01	0.07 ± 0.03[c]	>0.67 (0.88)	0.33 ± 0.09	gy
Petitgrain	0.49 ± 0.02	>0.67	0.036 ± 0.01	>0.67 (0.98)	0.21 ± 0.11	gy
Pine needle	>0.67	>0.67	0.059 ± 0.04	>0.67 (1.0)	0.35 ± 0.09	gy
Ravensara	0.40 ± 0.01	0.40 ± 0.06[e]	0.087 ± 0.06	>0.67	0.57 ± 0.02	gy

TABLE 3. *Continued*

Oil	BA50 value for: *E. coli* RM1484	*S. enterica* RM1309	*C. jejuni* RM1221	*L. monocytogenes* RM2199	*L monocytogenes* RM2388	Suspension[b]
Rose damask	0.55 ± 0.1[c]	0.44 ± 0.01	0.11 ± 0.03	0.55 ± 0.18	0.36 ± 0.07	y, r
Rose French	0.43 ± 0.02	0.50 ± 0.04	0.05 ± 0.04[c]	0.44 ± 0.25	0.29 ± 0.01	gy
Rose geranium	0.41 ± 0.02[f]	0.40 ± 0.01	0.088 ± 0.01[c]	0.60 ± 0.22	0.32 ± 0.01	gy
Rosemary	0.38 ± 0.01	0.45 ± 0.04	0.060 ± 0.02	>0.67 (1.4)	0.61 ± 0.01	gy
Rosewood	0.50 ± 0.11	0.43 ± 0.01	0.38 ± 0.06	>0.67 (0.75)	0.26 ± 0.01	gy
Sage clary	>0.67	>0.67	0.084 ± 0.001	>0.67 (1.0)	0.33 ± 0.21	gy
Sage white dalmatian	>0.67	>0.67	0.079 ± 0.10	>0.67 (1.2)	>0.67 (0.72)	gy
Sage white desert	>0.67	>0.67	0.070 ± 0.05	>0.67	0.31 ± 0.03	y
Sandalwood Indian	>0.67	>0.67	0.028 ± 0.04[p]	0.22 ± 0.12[f]	0.083 ± 0.07[f]	gy, r
Sassafras	>0.67	0.38 ± 0.21[c]	0.059 ± 0.04	0.38 ± 0.28	0.19 ± 0.06	gy
Sesame	>0.67	>0.67	>0.67	>0.67	>0.67	gy
Spearmint	0.28 ± 0.20	0.29 ± 0.15	0.030 ± 0.01[f]	0.31 ± 0.03	0.57 ± 0.22[f]	gy
Spikenard	>0.67	>0.67	0.009 ± 0.003	0.21 ± 0.32[c]	0.02 ± 0.01	y, gn
Spruce	>0.67	>0.67	0.10 ± 0.04	0.58 ± 0.10	0.44 ± 0.01	gy
Tangerine	>0.67	>0.67	0.10 ± 0.01	0.665 ± 0.04	0.20 ± 0.04	gy
Tarragon	>0.67 (1.6)	>0.67 (2.1)	0.017 ± 0.01[f]	>0.67 (1.3)	>0.67 (0.70)	gy
Tea tree	0.42 ± 0.01	0.18 ± 0.04	0.10 ± 0.005	0.41 ± 0.01	>0.67 (0.74)	gy
Thuja	>0.67	0.40 ± 0.04	0.10 ± 0.03	>0.67	>0.67	gy
Thyme	0.047 ± 0.001	0.045 ± 0.001	0.022 ± 0.002[f]	0.091 ± 0.03	0.22 ± 0.00	gy
Tuberose	>0.67	>0.67	0.015 ± 0.012[p]	>0.67	>0.67	gn
Vanilla oleo resin	>0.67	>0.67	0.10 ± 0	>0.67 (1.1)	>0.67 (0.80)	b
Wintergreen	>0.67	0.54 ± 0.12	0.24 ± 0.25	>0.67	0.11 ± 0.05[f]	gy
Wormwood	0.55 ± 0	0.52 ± 0.05	0.38 ± 0.02	0.50 ± 0.26	0.097 ± 0.006	b, gn
Ylang ylang	>0.67	>0.67	0.21 ± 0.11	0.65 ± 0.10	>0.67	b

[a] Values represent averages ± standard deviations for duplicate experiments unless noted otherwise. Values above BA50 of 0.67 are reported in parentheses.
[b] Characteristics of the 1% stock suspensions: b, brown; m, milky; gy, gray; gn, green; o, orange; y, yellow; r, rapid oil separation; s, soluble.
[c] $n = 3$ (correlation coefficient was <0.8 in one experiment).
[d] Based on single point (three others—BA50 > 0.67).
[e] $n = 3$ (one other—BA50 > 0.67).
[f] $n = 4$.
[g] $n = 18$, positive control.
[h] $n = 19$, positive control.
[i] $n = 15$, positive control.
[j] $n = 14$, positive control.
[k] $n = 12$, positive control.
[l] $n = 6$.
[m] $n = 7$, positive control.
[n] $n = 5$, positive control.
[o] $n = 6$, positive control.
[p] $n = 3$ (one other—too much kill for BA50 determination).

TABLE 4. *BA50 values for oil compounds against* Escherichia coli *O157:H7,* Salmonella enterica, Campylobacter jejuni, *and* Listeria monocytogenes[a]

Oil compound	BA50 value for: *E. coli* RM1484	*S. enterica* RM1309	*C. jejuni* RM1221	*L. monocytogenes* RM2199	*L. monocytogenes* RM2388	Oil source	Suspension[b]
Anethole, *trans*	>0.67	>0.67	0.12 ± 0.01	>0.67	>0.67 (0.90)	Anise	gy
Benzaldehyde	0.48 ± 0.01	0.36 ± 0.02[c]	0.019 ± 0.001	0.46 ± .0	0.36 ± .0	Bitter almond	gy
Bornyl acetate	>0.67	>0.67	0.10 ± 0.003	>0.67	>0.67 (0.92)	Many oils	gy
Carvacrol	0.057 ± 0.009	0.054 ± 0.01	0.011 ± 0.003	0.083 ± 0.01	0.086 ± 0.01	Thyme, oregano	gy, r
Carvone R	0.45 ± 0.004	0.41 ± 0.04	0.031 ± 0.005	>0.67	>0.67 (0.73)	Caraway seed	gn
Carvone S	0.48 ± 0.09	0.39 ± 0.02	0.044 ± 0.01	0.35 ± 0.01	0.17 ± 0.07	Dill seed	gn, gy
Cineol	>0.67	>0.67	0.10 ± 0.02	>0.67	>0.67	Dill seed	gy
Cinnamaldehyde	0.057 ± 0.034[d]	0.033 ± 0.014[e]	0.0028 ± 0.002[f]	0.019 ± 0.01	0.008 ± 0.001	Cinnamon	gy, m
Citral	0.22 ± 0.14[g]	0.23 ± 0.11[g]	0.021 ± 0.003	0.099 ± 0.005	0.20 ± 0.05	Lemon grass	gy, m
Citronella R	>0.67	>0.67	0.22 ± 0.11	>0.67 (1.2)	0.45 ± 0.10	Citronella	gy
Citronella S	>0.67	>0.67	0.050 ± 0.01	>0.67 (0.70)	0.44 ± 0.22	Lemon	gy
Estragole	0.28 ± 0.12	0.21 ± 0.07	0.004 ± 0.002	0.36 ± 0.01	0.35 ± 0.04	Tarragon	gn
Eugenol	0.11 ± 0.03[g]	0.087 ± 0.035[g]	0.022 ± 0.026[h]	0.061 (0.05)	0.081 ± 0.001	Clove	gn, r
Geraniol	0.15 ± 0.04[g]	0.15 ± 0.04[g]	0.10 ± 0.004	0.28 ± 0.01	0.51 ± 0.16	Rose	gy
Geranyl acetate	>0.67	>0.67	0.034 ± 0.01	>0.67 (0.77)	>0.67 (0.73)	Rose	gy
Isoeugenol	0.15 ± 0.02	0.16 ± 0.02	0.35 ± 0.06	>0.67 (0.77)	>0.67 (0.73)	Ylany ylang	gy, r
Limonene	>0.67	>0.67	0.35 ± 0.01	>0.67	0.25 ± 0.03	Lemon	gy
Linalool	0.40 ± 0.03	0.37 ± 0.04	0.35 ± 0	>0.67 (0.85)	>0.67 (0.68)	Cinnamon	gy
Menthol	0.53 ± 0.09	0.50 ± 0.06	0.40 ± 0.04	0.57 ± 0.03	0.48 ± 0.06	Peppermint	gy
Perillaldehyde	0.27 ± 0.17[g]	0.20 ± 0.13[g]	0.03 ± 0.001	0.35 ± 0.05	0.30 ± 0.02	Mandarin peel	gy, m
Salicylaldehyde	0.13 ± 0.01	0.12 ± 0.01	0.040 ± 004	0.43 ± 0.02	0.45 ± 0.1	Almond	y, gn
Terpineol	0.39 ± 0.04	0.18 ± 0.06	0.10 ± 0.001	0.56 ± 0.19	>0.67 (0.82)	Pine	gy, r
Thymol	0.060 ± 0.002	0.034 ± 0.002	0.024 ± 0.001	0.077 ± 0.01[g]	0.077 ± 0.02[g]	Thyme, oregano	gy

[a] Values represent averages ± standard deviations for duplicate experiments unless noted otherwise. Values above BA50 of 0.67 are reported in parentheses.
[b] Characteristics of the 1% stock suspensions: m, milky; gy, gray; gn, green; y, yellow; r, rapid oil separation.
[c] $n = 3$ (one other—BA50 > 0.67).
[d] $n = 15$.
[e] $n = 14$.
[f] $n = 12$.
[g] $n = 4$.
[h] $n = 1$ (one other—correlation coefficient was <0.8 in one experiment).

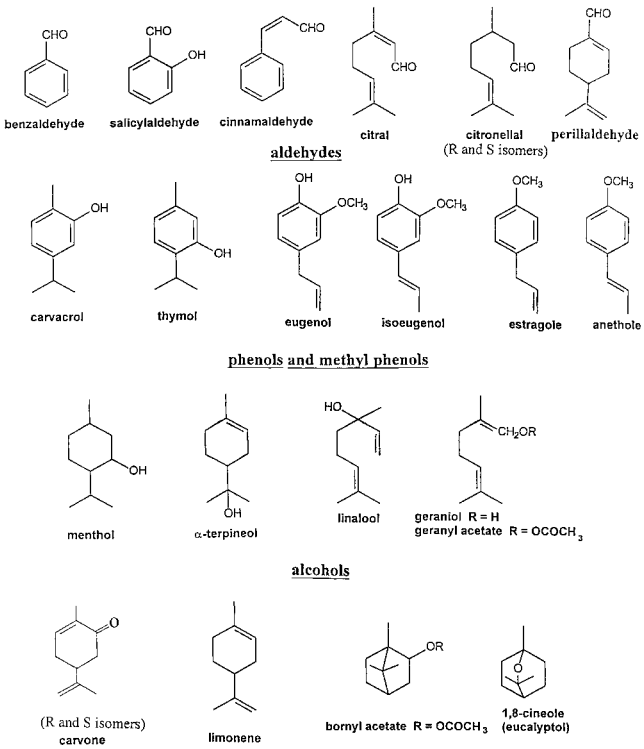

FIGURE 3. *Structures of 23 plant essential oil constituents evaluated in this study.*

Comparison of relative bactericidal activities of all compounds. The reciprocal bactericidal activities (1/BA50) of all compounds tested against the different strains (Tables 3 and 4) were plotted to compare the relative sensitivities among the strains (Fig. 4). The results presented in Figure 4 reveal the similar sensitivities of the *E. coli* O157:H7 and *S. enterica* strains, in contrast to the broad range of activities against *C. jejuni.*

DISCUSSION

We have shown that many plant essential oils and many compounds isolated from plants are bactericidal against multiple strains of *C. jejuni, E. coli* O157 and O157:H7, *L. monocytogenes,* and *S. enterica,* including strains associated with foodborne outbreaks, human illness, and recent food isolates. To facilitate an accurate comparison of the relative activities of plant compounds, we modified a bactericidal assay method previously described for gram-negative human mucosal pathogens *(27)* and developed a method for mixing and suspending oily plant-derived samples in aqueous buffers.

Sample preparation and assay methods. The ''shaking'' of samples to suspend the water-insoluble essential oils for bactericidal studies was done in a manner approximating what a processor might do as a final wash step before packaging or what a consumer might do to marinate a food prior to cooking or consumption. Moreover, since the bactericidal assay methods that have previously been described seem to differ considerably (see below), there has been a need for the development of a standardized assay to compare the antimicrobial activities of plant compounds.

Many types of assays for measuring the antimicrobial activities of plant-derived compounds have been described, and inconsistencies in results for different assay methods also have been reported. Types of assays described include the measurement of (i) the zone of inhibition of bacterial growth around paper disks containing a plant antimicrobial compound on tryptic soy agar *(15),* (ii) the minimum concentration necessary to inhibit the growth of bacteria (MIC) *(7, 10, 32),* (iii) the inhibition of bacterial growth on an agar medium with an antimicrobial compound diffused in the agar *(28),* and (iv) the comparative bacteriostatic activities of cinnamon and other oils as determined by an agar diffusion assay versus those as determined by a serial-dilution assay *(42).* The comparison of these latter methods by Yousef and Tawil *(42)* revealed that different results were obtained for the same antimicrobial compound in the different assays. Since such a difference is often a function of concentration, it is not always apparent whether the methods cited measure bacteriostatic activity, bactericidal activity, or perhaps a combination of both.

A number of previous studies are relevant to the theme of this paper. For example, it was shown that the size of an inoculum (number of bacteria) can influence the antibiotic activities of disinfectants against *Staphylococcus aureus (19).* The observed overestimation of disinfection by antimicrobial agents obtained with the so-called automated bioscreen compared with the estimate obtained with the traditional plate count method, which may be due to the extent of the injury incurred by microbes during the disinfection *(21, 23)* as well as the quenching of biocides by microbes during disinfection in a suspension test *(14),* suggests the need for improved methods of testing bactericidal activity. The observation that the level of antimicrobial effectiveness of oregano oil was lower in a gelatin matrix than in liquid culture suggests that diffusion influences activity *(36).* Antibiotic activity was also affected by interactions between the essential oil *Thymus vulgaris* and hydrophilic or hydrophobic components used in the formulation of cosmetics *(29).* Finally, kinetic equations can be used to describe and determine optimum rates of disinfection *(4, 20).*

The method described and used in this study is a microtiter plate–based bactericidal assay facilitating the screening of large numbers of plant-derived compounds in a suspension for antimicrobial activity against bacterial pathogens. The assay was easy to perform and the results were reproducible. The bacterial strains we tested had been isolated from patients or from retail plant or animal food products associated with human disease.

Dose-response activity in bactericidal assay for plant compounds. An examination of the activities of different concentrations of selected compounds against the prototype strains reveal that 1% concentrations of the most active compounds generally killed all of the bacteria within 30 min and that much lower concentrations killed 50% of the bacteria. The linear dose-response curves obtained with the different compounds and strains at kill rates of 20 to 70% were the basis for the selection of a kill rate of 50%

TABLE 5. *BA50 values for the 39 essential oils and oil compounds most active against all strains of bacteria tested*

Oil/oil compound	*E. coli* RM1484	*S. enterica* RM 1309	*C. jejuni* RM1221	*L. monocytogenes* RM2199	*L. monocytogenes* RM2388	Average	SD
Cinnamaldehyde	0.06	0.04	0.003	0.02	0.01	0.03	0.02
Thymol	0.06	0.03	0.02	0.08	0.08	0.05	0.02
Oregano Spanish	0.05	0.05	0.01	0.07	0.08	0.05	0.03
Carvacrol	0.06	0.05	0.01	0.08	0.09	0.06	0.03
Oregano origanum	0.05	0.05	0.02	0.08	0.10	0.06	0.03
Eugenol	0.11	0.09	0.02	0.06	0.08	0.07	0.03
Cinnamon leaf	0.13	0.08	0.03	0.09	0.09	0.08	0.04
Thyme	0.05	0.05	0.02	0.09	0.22	0.09	0.08
Bay leaf	0.13	0.13	0.03	0.07	0.07	0.09	0.04
Clove bud	0.13	0.13	0.02	0.07	0.09	0.09	0.05
Allspice	0.14	0.13	0.02	0.09	0.08	0.09	0.05
Cinnamon bark	0.18	0.14	0.02	0.09	0.08	0.10	0.06
Cinnamon cassia	0.11	0.07	0.01	0.19	0.15	0.11	0.07
Lemon grass	0.14	0.16	0.02	0.12	0.12	0.11	0.05
Palmarosa	0.12	0.14	0.07	0.17	0.27	0.15	0.07
Citral	0.22	0.23	0.02	0.10	0.20	0.15	0.09
Basil	0.41	0.42	0.02	0.09	0.12	0.21	0.19
Perillaldehyde	0.27	0.20	0.03	0.35	0.30	0.23	0.12
Salicylaldehyde	0.13	0.12	0.04	0.43	0.45	0.23	0.19
Geraniol	0.15	0.15	0.10	0.28	0.51	0.24	0.17
Estragole	0.28	0.21	0.01	0.36	0.35	0.24	0.14
Fir needle Siberian	0.48	0.61	0.01	0.13	0.08	0.26	0.27
Elemi	0.40	0.44	0.01	0.26	0.22	0.27	0.17
Orange mandarin	0.41	0.64	0.01	0.18	0.10	0.27	0.26
Cumin seed	0.30	0.36	0.10	0.37	0.25	0.28	0.11
Carvone S	0.48	0.39	0.04	0.35	0.17	0.29	0.18
Spearmint	0.28	0.29	0.03	0.31	0.57	0.30	0.19
Caraway	0.46	0.47	0.03	0.33	0.24	0.31	0.18
Citronella	0.41	0.49	0.09	0.40	0.18	0.31	0.17
Hyssop	0.57	0.41	0.10	0.33	0.18	0.32	0.19
Nutmeg	0.55	0.44	0.18	0.27	0.20	0.33	0.16
Benzaldehyde	0.48	0.36	0.02	0.46	0.36	0.34	0.19
Lavender	0.41	0.41	0.06	0.48	0.34	0.34	0.16
Rose French	0.43	0.50	0.05	0.44	0.29	0.34	0.18
Rose geranium	0.41	0.40	0.09	0.60	0.32	0.36	0.18
Rose damask	0.55	0.44	0.11	0.55	0.36	0.40	0.18
Wormwood	0.55	0.52	0.38	0.50	0.10	0.41	0.19
Coriander	0.40	0.48	0.08	0.67	0.50	0.43	0.22
Menthol	0.53	0.50	0.40	0.57	0.48	0.50	0.06

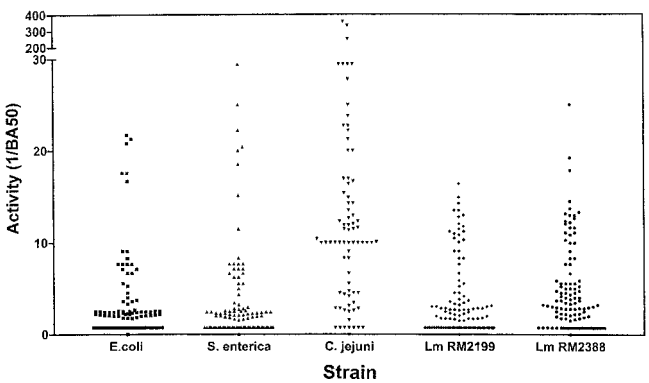

FIGURE 4. *Schematic representation of the bactericidal activities of all compounds against selected strains of* C. jejuni, E. coli *O157:H7,* L. monocytogenes, *and* S. enterica. *The BA50 values used in plotting this graph are shown in Tables 3 and 4.*

(i.e., BA50) for a comparison of the activities of multiple samples.

There is a need for a standard value with which to report bactericidal activity. We believe that the BA50 value could serve this purpose. It is based on the linear response of six concentrations inducing 100% destruction of bacteria. It should be noted that in almost all cases, concentrations of a compound that were higher than the BA50 value killed 100% of the bacteria. Moreover, BA50 values offer maximum precision with a dose-response analysis, because with regression analysis, maximum precision is achieved at the mean response, since the corresponding dose represents the center of the information upon which the design matrix is based (dose levels). The BA50 is analogous to the LD_{50} value widely used in animal studies.

Sensitivity differences among strains in bactericidal assay for plant compounds. Multiple strains of a pathogen

species can contaminate food. Therefore, it was of interest to determine the relative levels of susceptibility of different strains to different test compounds. The activities of a variety of the compounds appeared to be relatively similar for all of the strains represented within a species (Table 2). However, *C. jejuni* was approximately 5- to 10-fold more sensitive to many of the oils and oil compounds than were the other species. Our prototype *C. jejuni* strain, RM1221, had been isolated from chicken obtained at a retail market *(31).* Its levels of sensitivity to two essential oil samples and one oil compound sample appeared to be similar to those of the three other animal isolates of *C. jejuni* (Tables 1 and 2). The sensitivity levels of *C. jejuni* strains may be due to the fastidious growth conditions required by *C. jejuni* (e.g., microaerophilic atmosphere, rich medium) and perhaps also to differences in the outer membrane and cell wall structure of *C. jejuni* and those of the other gram-negative species and *L. monocytogenes.*

L. monocytogenes RM2199, which was isolated from cheese associated with a disease outbreak, appeared to be somewhat more resistant to compounds than was a strain of *L. monocytogenes* (RM2388) isolated from a store-bought mint. Although the reasons for the observed effects of strain on bactericidal activity against *L. monocytogenes* are not known, one possibility is that strain RM2199, which was isolated from infected cheese associated with a listeriosis outbreak *(1),* developed resistance while it was in the cheese and that strain RM2388, which was isolated from a retail mint, did not have the opportunity to develop such resistance. Another explanation may be that strain RM2199, which is an outbreak strain, has virulence factors not present in strain RM2388. It will be important to determine whether the resistance of strains to plant antimicrobial compounds is related to the source of the isolate or to the adaptation of the strain to environmental conditions (e.g., food surfaces and processing environments).

A comparison of our results and the results of previous studies of the antimicrobial activities of similar plant compounds reveals both agreement and differences. In a study of the antimicrobial activities of 52 essential oils against different microbial species, thyme oil was reported to be one of the most active oils against a strain of *E. coli,* with a reported MIC of 0.09% *(10).* This result is comparable to the BA50 value of 0.05 we report for the bactericidal activity of thyme oil against *E. coli* O157:H7 (Table 3). It was reported in the same study that bay leaf, lemon grass, and oregano oils at concentrations of 2.0% inhibited the growth of all organisms tested *(10).* This concentration is much higher than the BA50 values observed for the four pathogens tested in the present study for bay leaf (with BA50 values ranging from 0.034 to 0.13), lemon grass (with BA50 values ranging from 0.018 to 0.16), oregano origanum (with BA50 values ranging from 0.019 to 0.098), and thyme (with BA50 values ranging from 0.022 to 0.22) (Table 3).

In a related study, an agar well method was used to evaluate the antimicrobial activities of a number of plant compounds against *C. jejuni, S. enterica, E. coli, Staphylococcus aureus,* and *L. monocytogenes (37).* Cinnamon and thyme oils were reported to be active against all tested bacteria. The effective bacteriostatic and bactericidal concentrations were 0.23% and approximately 1%, respectively, for all of the strains except the *C. jejuni* strain, which was the most resistant of the five species tested. These results are consistent with our observations for *E. coli* O157:H7 strain RM1484 and *S. enterica* RM1309, but they are inconsistent with activities we report for multiple oil compounds against multiple *C. jejuni* strains being up to 100-fold more extensive than activities against the other three pathogens (Tables 3 through 5). These differences in results between studies could be explained by differences in the potencies or methods of preparation of oil stock samples, the sensitivities of strains, or the sensitivities of the assay methods and growth conditions. The effects of sample preparation on activity are illustrated by previous reports of (i) a 50-fold increase in the antimicrobial activity of cinnamon oil in the absence of the dispersing agent dimethyl sulfoxide *(12),* (ii) a decrease in the antimicrobial activity of various plant essential oils in the presence of the detergent Tween 80 and the solvent ethanol *(34),* and (iii) the enhancement of the antimicrobial activity of tea tree oil in a broth culture of *E. coli* supplemented with Tween 80.

Structure-function relationships. A number of associations can be discerned from comparisons of the chemical structures of the purified plant compounds (Fig. 3) and their antimicrobial activities (Table 4). For example, both the aldehyde compounds (e.g., cinnamaldehyde, citral, citronellal, perillaldehyde, and salicylaldehyde) and the phenolic compounds (e.g., carvacrol, eugenol, isoeugenol, and thymol) were very active in our bactericidal assay. However, we noted that the activities of two pairs of isomeric compounds, eugenol-isoeugenol and anethole-estragole, each of which differ only with regard to the position of the double bond in the aliphatic side chain, were significantly different against certain strains. Eugenol was about 13-fold more active than isoeugenol against *C. jejuni* and *Listeria,* whereas the activities of the two compounds against *E. coli* and *S. enterica* were similar (with BA50 values of 0.11 and 0.087, respectively). Estragole was highly active against *C. jejuni* (BA50 = 0.004) and only moderately active (with BA50 values ranging from 0.21 to 0.36) against the other species tested. In contrast, the estragole isomer anethole was 30-fold less active against *C. jejuni* (BA50 = 0.12) and inactive (i.e., BA50 > 0.67) against the other species. Carvacrol and thymol, which differ with regard to the position of the single OH group, exhibited similar activities against all of the strains tested.

Monoterpenes with an exocyclic double bond (eugenol and estragole) were generally more active than the isomers with endocyclic double bonds (anethole and isoeugenol); in contrast, the 2-carvone optical isomers (*R* and *S*) were equally active against *E. coli, S. enterica,* and *C. jejuni* prototype strains, but there was a two- to threefold difference in their levels of activity against the two *L. monocytogenes* strains (with *S* being more active than *R*). The two optical isomers of citronellal were inactive against *E. coli, S. enterica,* and *L. monocytogenes* strain RM2199, but their

activities against *L. monocytogenes* RM2388 were identical (with BA50 values of 0.45 and 0.44) and there was a fourfold difference in their activities against *C. jejuni* (with BA50 values of 0.050 and 0.22 for *S* and *R,* respectively). These results extend the more limited findings of Kim et al. *(15, 16),* who used a paper-disk method to assess the MICs and bactericidal concentrations of several oil compounds against *E. coli* O157:H7, *Salmonella* Typhimurium, *L. monocytogenes,* and *Vibrio vulnificus.* Carvacrol, citral, citronellal, geraniol, linalool, perillaldehyde, and terpineol were bactericidal at around 100 μg/ml. Possible reasons for the differences and similarities in reported activities among isomers are not known.

C. jejuni strains were more sensitive to most of the compounds than were strains of other species, as noted above. The prototype *C. jejuni* strain was 100-fold more sensitive to some compounds than were strains of the other three species. Gardenia oil, which was inactive against *E. coli* and *S. enterica* at the concentrations tested, was highly active against *C. jejuni* (BA50 = 0.007) and *L. monocytogenes* RM2388 (BA50 = 0.038). Moreover, *C. jejuni* was much more sensitive to almond bitter oil, benzaldehyde, and salicylaldehyde than were the other three species. This sensitivity may be due to the strict conditions required by *C. jejuni* to grow and the possible inhibition by oils and oil compounds of metabolic pathways important for growth. Alternatively, it may reflect damaged membranes and decreased *C. jejuni* viability resulting from the outer surface characteristics of *C. jejuni,* which are different from those of the other species.

Chemical analysis to determine the ratios of active compounds in oils from different sources has previously been described *(8a).* Cinnamon bark oil was shown to contain 62% *trans*-cinnamaldehyde and no eugenol; in contrast, cinnamon cassia oil was found to contain 81% *trans*-cinnamaldehyde and no eugenol, and cinnamon leaf oil was found to contain 70% eugenol but no cinnamaldehyde *(8a).* Our bactericidal data for these three oils (Table 3) indicate that cinnamon bark oil was 25% to 50% as active against *E. coli* and *S. enterica* as the other two cinnamon oils but that the activities of the three oils against *C. jejuni* were similar. The cinnamon bark and cinnamon leaf oils exhibited similar activities against both *Listeria* strains and were about twice as active against these strains as the cassia oil. Our results indicate that the activities of cinnamaldehyde and eugenol tended to correlate directly with the activities of the oils from which they were derived. This conclusion is reinforced by the related observation that the inhibition of the growth of *S. aureus* and *Pseudomonas aeruginosa* by oregano oil is due to the carvacrol and thymol present in this oil and that inhibitory effects of these two compounds are additive *(22).* In addition, our results suggest that plants from different regions and different samples of a plant can contain markedly different amounts of active compounds and that it may be preferable to use synthetic oil compounds rather than the more expensive oils in food applications.

Potential applications of essential oils and their compounds to food. The nature of the plants from which some essential oils have been derived necessitates consideration of the sensory properties (e.g., flavor, aroma, color) of compounds to be added to food or water. The flavors of oils can vary widely (e.g., spicy cinnamon oil *(8a)* versus mild oregano oil); therefore, some oils would be more appropriate than others for foods such as fresh produce, juices, dairy products, poultry, and red meat. Also, the antimicrobial activities of essential oils under conditions associated with such processes as baking, cheesemaking, cooking, frying, fruit juice preparation, and microwaving are mostly unknown. Therefore, there is a need to determine changes in antimicrobial properties under these conditions.

Several reports on the antimicrobial effectiveness of essential oils in foods suggest that the use of these oils may improve food safety. For example, Koutsoumanis et al. *(17)* inoculated a salad with several concentrations of oregano oil and with *Salmonella* Enteritidis and then evaluated the influence of several environmental factors on the microbial population. The death rate of the pathogen depended on the pH, the storage temperature and time, and the essential oil concentration. In a related study, Tassou et al. *(40)* reported that *Salmonella* Enteritidis inoculated into a gilt-head seabream dressed with olive oil, lemon juice, and oregano oil survived much better in an aerobic atmosphere than in an anaerobic atmosphere, whereas the coinoculated *S. aureus* population showed equal reductions in both atmospheres. Lin et al. *(25)* showed that vapors generated from plant-derived isothiocyanates resulted in an 8-log reduction in *E. coli* O157:H7 and *Salmonella* Montevideo on iceberg lettuce and tomato skins inoculated with these pathogens, although the same treatment was less effective by approximately 5 log units for inoculated apple stem scars. Skandamis and Nychas *(35)* found that oregano oil inhibited the microbial growth of spoilage organisms in minced meat; it also changed physicochemical properties of the meat. The summary of activities of the most active oils for all of the strains tested (Table 5) will be helpful in devising antimicrobial formulations with which to protect foods against infection by multiple pathogens.

Potential mechanisms of antimicrobial activities. It is unlikely that the observed antimicrobial activities of the oils and/or the active components are due to a physical phenomenon such as aggregation or concentration of the bacteria. For example, several of the oils were inactive against each of the species tested. In addition, the concentration dependence of antimicrobial activity argues against a nonspecific mechanism of antimicrobial activity. The structure-function analysis of the compounds tested in this study has established clues to aid in the determination of the mechanisms of antimicrobial activities against the bacteria studied here. However, detailed examination of possible mechanisms involving inhibition of essential enzymes, chelation of essential trace elements such as iron, interference with cell membrane biosynthesis, and disruption of cell membranes are beyond the scope of this paper *(2, 8).* These aspects, as well as the reasons for the observed differences in the susceptibility levels of different strains of the same microorganism (Fig. 4), merit study.

CONCLUSIONS

We examined the antimicrobial activities of a variety of oils and oil compounds against *C. jejuni, E. coli, L. monocytogenes,* and *S. enterica* strains as well as some of the factors that may influence these activities. We find it striking that strains of *E. coli, L. monocytogenes,* and *S. enterica* exhibit similar susceptibilities to inactivation by both essential oils and oil compounds. In contrast, the susceptibility of *C. jejuni* to inactivation by many of the same natural compounds was much greater than those of enteric bacteria and *L. monocytogenes.* The shaking technique used for sample application, which mimics the application of some of the oils to foods, was easy to perform. To facilitate the comparison of results obtained by different investigators, it is important that oil compounds be suspended thoroughly before bacteria are added for the testing of antimicrobial activity, and assays should generally be carried out under conditions relevant to the environments in which microbes would be encountered. The ultimate goal of these studies is to develop safe, effective, and inexpensive food formulations and processes to reduce pathogens in food. The antimicrobial compounds identified in this study as the most active against four major foodborne pathogens are candidates for future studies of synergism, compatibility, and activity in foods or food-processing systems and mechanisms of activity for specific pathogens.

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